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(54) Title: METHOD OF INHIBITING FIBROSIS WITH A SOMATOSTATIN AGONIST

(57) Abstract

The present invention relates to a method of inhibition fibrosis in a patient. The method includes the step of administering a therapeutically effective amount of a somatostatin or a somatostatin agonist to said patient.

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METHOD OF INHIBITING FIBROSIS WITH A SOMATOSTATIN AGONIST

Background of the Invention

Tissue comprises organized cellular groups that are attached to an extracellular matrix and are surrounded by a network of blood vessels. Fibrosis is an abnormal accumulation of connective fibrous tissue (e.g., an extracellular collagen matrix) following injury or inflammation which alters the structure and function of various tissues. Irrespective of location, the major pathology of fibrosis involves an excessive deposition of a collagen matrix which replaces the normal tissue at that site. Progressive fibrosis in the kidney, liver, lung, heart, bone marrow, and skin is a major cause of death and suffering. See, e.g., Border, et al., New Engl. J. Med. 331:1286 (1994).

Summary of the Invention

The present invention relates to a method of treating fibrosis in a patient (e.g., a mammal such as a human). The method includes the step of administering a therapeutically effective amount of somatostatin or a somatostatin agonist to said patient. The somatostatin or somatostatin agonist may be administered parenterally, e.g., administered intravenously, subcutaneously, or by implantation of a sustained release formulation. Fibrosis is the abnormal accumulation of connective fibrous tissue (e.g., an extracellular collagen matrix) in the body. The fibrosis, for example, may be located in the kidney (e.g., fibrosis as observed in glomerulonenephritis, diabetic nephropathy, allograft rejection, and HIV nephropathy), liver (e.g., fibrosis as observed in cirrhosis and veno-occlusive disease), lung (e.g., idiopathic fibrosis, chemotherapy induced

fibrosis, and autoimmune induced fibrosis), skin (e.g., systemic sclerosis, keloids, scars, and eosinophilia-myalgia syndrome), central nervous system (e.g., intraocular fibrosis), or nose (e.g., nasal polyposis).

Definition of "somatostatin agonist" will be defined below. A therapeutically effective amount depends upon the condition being treated, the route of administration chosen, and the specific activity of the compound used and ultimately will be decided by the attending physician or veterinarian. In one embodiment, the somatostatin agonist is administered to the patient until the fibrotic process is arrested and/or is reversed. In another embodiment, the somatostatin agonist is administered for the lifetime of the patient. In still another embodiment, the somatostatin agonist is administered prior to the event which initiates the fibrotic process (e.g., prior to chemotherapy).

The somatostatin agonist may be injected parenterally, e.g., intravenously, into the bloodstream of the subject being treated. However, it will be readily appreciated by those skilled in the art that the route, such as subcutaneous, intramuscular, intraperitoneal, enterally, transdermally, transmucously, sustained released polymer compositions (e.g., a lactic acid polymer or lactic-glycolic acid copolymer microparticle or implant), profusion, nasal, oral, etc., will vary with the condition being treated and the activity and bioavailability of the somatostatin agonist being used.

While it is possible for the somatostatin agonist to be administered as the pure or substantially pure compound, it may also be presented as a pharmaceutical formulation or preparation. The formulations to be used in the present invention, for both humans and animals, comprise any of the somatostatin agonists to be described

below, together with one or more pharmaceutically acceptable carriers thereof, and ptionally other therapeutic ingredients.

The carrier must be "acceptable" in the sense of being compatible with the active ingredient(s) of the formulation (e.g., capable of stabilizing peptides) and not deleterious to the subject to be treated. Desirably, the formulation should not include oxidizing agents or other substances with which peptides are known to be incompatible. For example, somatostatin agonists in the cyclized form (e.g., internal cysteine disulfide bond) are oxidized; thus, the presence of reducing agents as excipients could lead to an opening of the cysteine disulfide bridge. On the other hand, highly oxidative conditions can lead to the formation of cysteine sulfoxide and to the oxidation of tryptophane. Consequently, it is important to carefully select the excipient. pH is another key factor, and it may be necessary to buffer the product under slightly acidic conditions (pH 5 to 6).

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient(s) into association with the carrier which constitutes one or more accessory ingredients.

In general, the formulations for tablets or powders are prepared by uniformly and intimately blending the active ingredient with finely divided solid carriers, and then, if necessary, as in the case of tablets, forming the product into the desired shape and size.

Formulations suitable for parenteral (e.g., intravenous) administration, on the other hand, conveniently comprise sterile aqueous solutions of the active ingredient(s). Preferably, the solutions are isotonic with the blood of the subject to be treated. Such formulations may be conveniently prepared by

dissolving solid active ingredient(s) in water to produce an aqueous solution, and rendering said solution sterile. The formulation may be presented in unit or multi-dose containers, for example, sealed ampoules or vials.

Formulations suitable for sustained release parenteral administrations (e.g., biodegradable polymer formulations) are also well known in the art. See, e.g., U.S. Patent Nos. 3,773,919 and 4,767,628 and PCT Publication No. WO 94/15587.

The somatostatin or somatostatin agonist may also be administered with known initiators (e.g., chemotherapeutics) of the fibrotic process to prevent the initiation of fibrosis.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments and from the claims.

Abbreviations

β-Nal = β-naphthylalanine

β-Pal = β-pyridylalanine

hArg(Bu) = N-guanidino-(butyl)-homoarginine

$hArg(Et)_2$ = N, N'-guanidino-(dimethyl)-homoarginine

$hArg(CH_2CF_3)_2$ = N, N'-guanidino-bis-(2,2,2,-trifluoroethyl)-homoarginine

hArg(CH_3, hexyl) = N, N'-guanidino-(methyl, hexyl)-homoarginine

Lys(Me) = N^{ϵ}-methyllysine

Lys(iPr) = N^{ϵ}-isopropyllysine

AmPhe = aminomethylphenylalanine

AChxAla = aminocyclohexylalanine

Abu = α-aminobutyric acid

Tpo = 4-thiaproline

MeLeu = N-methylleucine

Orn = ornithine

Nle = norleucine

Nva = norvaline

Trp(Br) = 5-bromo-tryptophan
Trp(F) = 5-fluoro-tryptophan
Trp(NO_2) = 5-nitro-tryptophan
Gaba = γ-aminobutyric acid
Bmp = β-mercaptopropionyl
Ac = acetyl
Pen = pencillamine

Detailed Description of the Invention

It is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference.

Somatostatin and its Agonists

Somatostatin (somatotropin release inhibiting factor or SRIF) has both a 14 amino acid isoform (somatostatin-14) and a 28 amino acid isoform (somatostatin-28). See Wilson, J. & Foster, D., *Williams Textbook of Endocrinology*, p. 510 (7th ed., 1985). The compound is an inhibitor of secretion of the growth hormone and was originally isolated from the hypothalamus. Brazeau et al., Science 179:77 (1973). Native somatostatin has a very short duration of effect *in vivo* since it is rapidly inactivated by endo- and exopeptidase. Many novel analogs have been prepared in order to enhance the duration of effect, biological activity, and selectivity (e.g., for the particular

somatostatin receptor) of this hormone. Such analogs will be called "somatostatin agonists" herein.

Various somatostatin receptors (SSTRs) have been isolated, e.g., SSTR-1, SSTR-2, SSTR-3, SSTR-4, and SSTR-5. Thus, the somatostatin agonist may be a SSTR-1 agonist, SSTR-2 agonist, SSTR-3 agonist, SSTR-4 agonist of a SSTR-5 agonist. In one embodiment, the somatostatin agonist is an SSTR-2 agonist or an SSTR-5 agonist. What is meant by an "SSTR-2 agonist" or an "SSTR-5 agonist" is a compound which (1) has a high affinity (e.g., Ki of less than 1 μM or, preferably, of less than 10 nM) for the SSTR-2 or SSTR-5, respectively (as defined by the receptor binding assay described below), and (2) inhibits the formation of fibrosis (e.g., as defined by the biological assay described below). The somatostatin agonist may also be selective for a particular somatostatin receptor, e.g., have a higher binding affinity for a particular somatostatin receptor subtype. In one embodiment, the somatostatin receptor is an SSTR-2 or SSTR-5 selective agonist.

Somatostatin agonists which can be used to practice the therapeutic method of the present invention include, but are not limited to, those covered by formulae or those specifically recited in the publications set forth below, all of which are hereby incorporated by reference.

EP Application No. P5 164 EU (Inventor: G. Keri);

Van Binst, G. et al. Peptide Research 5:8 (1992);

Horvath, A. et al. Abstract, "Conformations of Somatostatin Analogs Having Antitumor Activity", 22nd European peptide Symposium, September 13-19, 1992, Interlaken, Switzerland;

PCT Application WO 91/09056 (1991);

EP Application 0 363 589 A2 (1990);

U.S. Patent No. 4,904,642 (1990);

U.S. Patent No. 4,871,717 (1989);

U.S. Patent No. 4,853,371 (1989);

U.S. Patent No. 4,725,577 (1988);
U.S. Patent No. 4,684,620 (1987)
U.S. Patent No. 4,650,787 (1987);
U.S. Patent No. 4,603,120 (1986);
U.S. Patent No. 4,585,755 (1986);
EP Application 0 203 031 A2 (1986);
U.S. Patent No. 4,522,813 (1985);
U.S. Patent No. 4,486,415 (1984);
U.S. Patent No. 4,485,101 (1984);
U.S. Patent No. 4,435,385 (1984);
U.S. Patent No. 4,395,403 (1983);
U.S. Patent No. 4,369,179 (1983);
U.S. Patent No. 4,360,516 (1982);
U.S. Patent No. 4,358,439 (1982);
U.S. Patent No. 4,328,214 (1982);
U.S. Patent No. 4,316,890 (1982);
U.S. Patent No. 4,310,518 (1982);
U.S. Patent No. 4,291,022 (1981);
U.S. Patent No. 4,238,481 (1980);
U.S. Patent No. 4,235,886 (1980);
U.S. Patent No. 4,224,190 (1980);
U.S. Patent No. 4,211,693 (1980);
U.S. Patent No. 4,190,648 (1980);
U.S. Patent No. 4,146,612 (1979); and
U.S. Patent No. 4,133,782 (1979).

Examples of somatostatin agonists include, but are not limited to, the following somatostatin analogs which are disclosed in the above-cited references:

D-β-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH2 (BIM-23014);
D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-β-Nal-NH2;
D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-β-Nal-NH2;
D-β-Nal-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH2;
D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH2;
D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr-NH2;
D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-OH;
D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr-OH;

Gly-Pen-Phe-D-Trp-Lys-Thr-Cys-Thr-OH;

Phe-Pen-Tyr-D-Trp-Lys-Thr-Cys-Thr-OH;

Phe-Pen-Phe-D-Trp-Lys-Thr-Pen-Thr-OH;

H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol;

H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

H-D-Trp-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Trp-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

Ac-D-Phe-Lys*-Tyr-D-Trp-Lys-Val-Asp-Thr-NH_2 (an amide bridge formed between Lys* and Asp);

Ac-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(Bu)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(Et)$_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-L-hArg(Et)$_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(CH_2CF_3)$_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(CH_2CF_3)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(CH_2CF_3)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH_2;

Ac-D-hArg(CH_2CF_3)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;

Ac-L-hArg(CH_2-CF_3)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(CH_2CF_3)$_2$-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-Cys-Thr-NH_2;

Ac-D-hArg(CH_2CF_3)$_2$-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-Cys-Thr-NHEt;

Ac-hArg(CH_3, hexyl)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

H-hArg(hexyl$_2$)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;

Ac-D-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH_2;

Propionyl-D-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys(iPr)-Thr-Cys-Thr-NH_2;

Ac-D-β-Nal-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Gly-hArg(Et)$_2$-NH_2;

Ac-D-Lys(iPr)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(CH_2CF_3)$_2$-D-hArg(CH_2CF_3)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(CH_2CF_3)$_2$-D-hArg(CH_2CF_3)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH_2;

Ac-D-hArg(Et)$_2$-D-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-Cys-Lys-Asn-4-Cl-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys-NH_2;

Bmp-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

Bmp-Tyr-D-Trp-Lys-Val-Cys-Phe-NH_2;

Bmp-Tyr-D-Trp-Lys-Val-Cys-p-Cl-Phe-NH_2;

Bmp-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH_2;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-β-Nal-NH_2;

H-pentafluoro-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

Ac-D-β-Nal-Cys-pentafluoro-Phe-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH_2;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

H-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

Ac-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

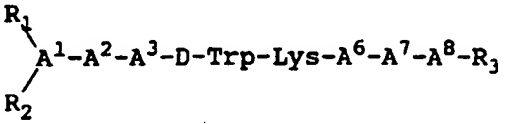
H-D-Phe-Cys-β-Nal-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2;
cyclo (Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);
cyclo (Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);
cyclo (Pro-Phe-D-Trp-Lys-Thr-N-Me-Phe);
cyclo (N-Me-Ala-Tyr-D-Trp-Lys-Thr-Phe);
cyclo (Pro-Tyr-D-Trp-Lys-Thr-Phe);
cyclo (Pro-Phe-D-Trp-Lys-Thr-Phe);
cyclo (Pro-Phe-L-Trp-Lys-Thr-Phe);
cyclo (Pro-Phe-D-Trp(F)-Lys-Thr-Phe);
cyclo (Pro-Phe-Trp(F)-Lys-Thr-Phe);
cyclo (Pro-Phe-D-Trp-Lys-Ser-Phe);
cyclo (Pro-Phe-D-Trp-Lys-Thr-p-Cl-Phe);
cyclo (D-Ala-N-Me-D-Phe-D-Thr-D-Lys-Trp-D-Phe);
cyclo (D-Ala-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Phe);
cyclo (D-Ala-N-Me-D-Phe-D-Thr-Lys-D-Trp-D-Phe);
cyclo (D-Abu-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Tyr);
cyclo (Pro-Tyr-D-Trp-t-4-AchxAla-Thr-Phe);
cyclo (Pro-Phe-D-Trp-t-4-AchxAla-Thr-Phe);
cyclo (N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe);
cyclo (N-Me-Ala-Tyr-D-Trp-t-4-AchxAla-Thr-Phe);
cyclo (Pro-Tyr-D-Trp-4-Amphe-Thr-Phe);
cyclo (Pro-Phe-D-Trp-4-Amphe-Thr-Phe);
cyclo (N-Me-Ala-Tyr-D-Trp-4-Amphe-Thr-Phe);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba-Gaba);
cyclo (Asn-Phe-D-Trp-Lys-Thr-Phe);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-$NH(CH_2)_4CO$);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-β-Ala);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-D-Glu)-OH;
cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe);
cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);
cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);
cyclo (Asn-Phe-Phe-D-Trp(F)-Lys-Thr-Phe-Gaba);
cyclo (Asn-Phe-Phe-D-Trp(NO_2)-Lys-Thr-Phe-Gaba);
cyclo (Asn-Phe-Phe-Trp(Br)-Lys-Thr-Phe-Gaba);
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe(I)-Gaba);

cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Tyr(But)-Gaba);
cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Pro-Cys)-OH;
cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Pro-Cys)-OH;
cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Tpo-Cys)-OH;
cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-MeLeu-Cys)-OH;
cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-Phe-Gaba);
cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-D-Phe-Gaba);
cyclo (Phe-Phe-D-Trp(5F)-Lys-Thr-Phe-Phe-Gaba);
cyclo (Asn-Phe-Phe-D-Trp-Lys(Ac)-Thr-Phe-NH-$(CH_2)_3$-CO);
cyclo (Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo (Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo (Orn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba); and
H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH_2(BIM-23268).

Note that for all somatostatin agonists described herein, each amino acid residue represents the structure of
-NH-C(R)H-CO-, in which R is the side chain (e.g., CH_3 for Ala) except for Thr-ol which means -NH-CH(CH(CH_3)OH)-CH_2-OH and Pro which means prolinyl. Lines between amino acid residues represent peptide bonds which join the amino acids. Also, where the amino acid residue is optically active, it is the L-form configuration that is intended unless D-form is expressly designated. A disulfide bridge is formed between two Cys residues; however, it is not shown.

Use of linear somatostatin agonists of the following formula is also within the invention:



wherein

A^1 is a D- or L- isomer of Ala, Leu, Ile, Val, Nle, Thr, Ser, β-Nal, β-Pal, Trp, Phe, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^2 is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^3 is pyridyl-Ala, Trp, Phe, β-Nal, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^6 is Val, Ala, Leu, Ile, Nle, Thr, Abu, or Ser;

A^7 is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^8 is a D- or L-isomer of Ala, Leu, Ile, Val, Nle, Thr, Ser, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

each R_1 and R_2, independently, is H, lower acyl or lower alkyl; and R_3 is OH or NH_2; provided that at least one of A^1 and A^8 and one of A^2 and A^7 must be an aromatic amino acid; and further provided that A^1, A^2, A^7 and A^8 cannot all be aromatic amino acids.

Examples of linear agonists to be used in the method of this invention include:

H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Thr-Phe-Thr-NH_2;

H-D-Phe-p-NO_2-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

H-D-Nal-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

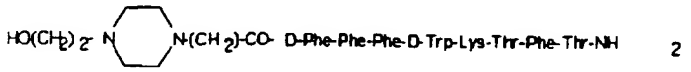
H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2;

H-D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2; and

H-D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-β-D-Nal-NH_2.

If desired, one or more chemical moieties, e.g., a sugar derivative, mono or poly-hydroxy C_{2-12} alkyl, mono or poly-hydroxy C_{2-12} acyl groups, or a piperazine derivative, can be attached to the somatostatin agonist, e.g., to the N-terminus amino acid. See PCT Application WO 88/02756, European Application 0 329 295, and PCT Application No. WO 94/04752. An example of a somatostatin agonists which contain N-terminal chemical substitutions are:



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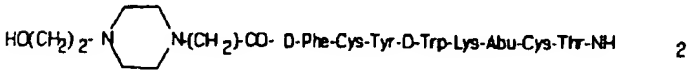
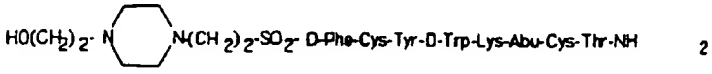


(BIM-23190); and



(BIM-23197).

Synthesis of somatostatin agonists

The methods for synthesizing somatostatin agonists are well documented and are within the ability of a person of ordinary skill in the art.

Synthesis of short amino acid sequences is well established in the peptide art. For example, synthesis of D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, described above, can be synthesized by following the protocol set forth in U.S. Patent No. 4,853,371 and synthesis of H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2, described above, can be achieved by following the protocol set forth in Example I of European Patent Application 0 395 417 A1. The synthesis of somatostatin agonists with a substituted N-terminus can be achieved, for example, by following the protocol set forth in WO 88/02756, European Patent Application No. 0 329 295, and PCT Publication No. WO 94/04752.

Somatostatin Receptor Binding Assays

The human SSTR-1, SSTR-2, SSTR-3, SSTR-4, and SSTR-5 cDNA clones have been described (SSTR-1 and SSTR-2 in Yamada, Y., et al., Proc. Natl. Acad. Sci. USA., 89:251-255 (1992); SSTR-3 in Yamada, et al., Mol. Endocrinol. 6:2136-2142 (1993); and SSTR-4 and SSTR-5 in Yamada, et al., Biochem. Biophys. Res. Commun. 195:844-852 (1993)) and are also available from American Type Culture Collection (ATCC, Rockville, MD) (ATCC Nos. 79044 (SSTR-1), 79046 (SSTR-2), and 79048 (SSTR-3)). Based on the restriction endonuclease maps, the entire coding region of each SSTR cDNA may be excised by suitable restriction endonuclease digestion (Maniatis, T., et al., *Molecular Cloning - A Laboratory Manual*, CSHL, 1982). Restriction endonucleases are available from New England Biolabs (Beverly, MA). This cDNA fragment was inserted into the mammalian expression vector, pCMV (Russell, D., et al., J. Biol. Chem., 264:8222-8229 (1989)), using standard molecular biology techniques (see e.g.,

Maniatis, T., et al., Molecular Cloning,-A Laboratory Manual, Cold Spring Harbor Laboratory, 1982) to produce the expression plasmid, pCMV-human SSTR-1 through pCMV-human SSTR-5. Other mammalian expression vectors include pcDNA1/Amp (Invitrogen, Sandlesy, CA). The expression plasmids were introduced into the suitable bacterial host, E. Coli HB101 (Stratagene, La Jolla, CA) and plasmid DNAs, for transfection, were prepared on Cesium Chloride gradients.

CHO-K1 (ovary, Chinese hamster) cells were obtained from ATCC (ATCC No. CCL 61). The cells were grown and maintained in Ham's F12 media (Gibco BRL, Grand Island, NY) supplemented with 10% fetal bovine serum under standard tissue culture conditions. For transfection, the cells were seeded at a density 1 x 10^6/60-cm plate (Baxter Scientific Products, McGaw Park, IL.). DNA mediated transfection was carried out using the calcium phosphate co-precipitation method (Ausubel, F.M., et al., Current Protocols in Molecular Biology, John Wiley & Sons, 1987). The plasmid pRSV-neo (ATCC; ATCC No. 37198) was included as a selectable marker at 1/10 the concentration of the expression plasmid. CHO-K1 clonal cell lines that have stably inherited the transfected DNA were selected for growth in Ham's F12 media containing 10% fetal bovine serum and 0.5mg/ml of G418 (Sigma). The cells were ring-cloned and expanded in the same media for analysis.

Expression of the human SSTR-1 through SSTR-5 receptors in the CHO-K1 cells were detected by Northern blot analysis of total RNA prepared from the cells (Sambrook, J.E., et al., Molecular Cloning - A Laboratory Manual, Ed. 2., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989) and by receptor binding using [^{125}I-Tyr^{11}]somatostatin-14 as a ligand. Transfected cell lines expressing the human SSTR receptors were clonally expanded in culture and used in the following SSTR binding protocol.

Crude membranes were prepared by homogenization of the transfected cells in 20 ml of ice-cold 50 mM Tris-HCl with a POLYTRON homogenizer (setting 6, 15 sec). Buffer was added to obtain a final volume of 40 ml, and the homogenate was centrifuged in a Sorval SS-34 rotor at 39,000 g for 10 min at 0-4°C. The resulting supernatant was decanted and discarded. The pellet was rehomogenized in ice-cold buffer, diluted, and centrifuged as before. The final pellet was resuspended in the 10 mM Tris HCl and held on ice for the receptor binding assay.

Aliquots of the membrane preparation were incubated for 30 min at 30°C with 0.05 nM [^{125}I-Tyr^{11}]somatostatin-14 (2000 Ci/mmol; Amersham Corp., Arlington Heights, IL) in 50 mM HEPES (pH 7.4) containing a test somatostatin agonist of various concentrations (e.g., 10^{-11} to 10^{-6}), 10 mg/ml bovine serum albumin (fraction V) (Sigma Chemical Co., St. Louis, MO), $MgCl_2$ (5 mM), Trasylol (200 KIU ml), bacitracin (0.02 mg/ml), and phenylmethylsulphonyl fluoride (0.02 mg/ml). The final assay volume was 0.3 ml. The incubations were terminated by rapid filtration through GF/C filters (pre-soaked in 0.3% polyethylenimine for 30 min) using a Brandel filtration manifold. Each tube and filter were then washed three times with 5 ml aliquots of ice-cold buffer. Specific binding was defined as the total [^{125}I-Tyr^{11}]somatostatin-14 bound minus that bound in the presence of 1000 nM of somatostatin-14. The Ki values for the tested somatostatin agonists were calculated by using the following formula: $Ki = IC_{50} / [1+(LC/LEC)]$ where IC_{50} is the concentration of test somatostatin agonist required to inhibit 50 percent of the specific binding of the radioligand [^{125}I-Tyr^{11}]somatostatin-14, LC is the concentration of the radioligand (0.05 nM), and LEC is the equilibrium dissociation constant of the radioligand (0.16 nM). The Ki values for the tested somatostatin agonists are shown in Table I.

TABLE I

	hSSTR-1	hSSTR-	hSSTR-3	hSSTR-	hSSTR-
Somatostatin -14	2.256	0.71	1.432	1.768	0.883
Somatostatin -28	2.382	0.57	1.021	7.93	0.383
BIM-23014	2414	1.10	121	1826	5.21
BIM-23190	5210	0.47	2154	7537	11.1
BIM-23197	6016	0.09	26.8	3897	9.81
BIM-23268	12.27	6.84	62	19.96	0.38

Inhibition of Fibrosis

The somatostatin agonists may be tested for their ability to inhibit fibrosis.

(a) Demonstration of Anti-Fibrotic Activity *In Vitro*

Rats are injected either with anti-thymocyte serum (ATS) to induce glomerulonephritis or with phosphate buffered saline (PBS) to serve as controls. Six days later, the kidneys are removed, and the glomeruli are isolated and placed in culture for 72 hours. Culture conditions consist of 2000 glomeruli/well in a 1 ml volume of serum-free RPMI 1640 (with insulin supplementation). Test somatostatin or somatostatin agonists are added at the time of culture. The supernatant from the cultures is collected and stored at -70ºC until assayed to determine the concentration of collagen I, transforming growth factor β-1 (TGFβ-1), fibronectin containing an extra domain A (fibronectin EDA+), and plasminogen activator inhibitor I (PAI-I) as markers of fibrotic activity. In addition, individual glomeruli are examined by immunofluorescent staining and scored for relevant matrix proteins. Values were

compared between PBS-treated, negative fibrotic control glomeruli; ATS-treated, non-drug treated, positive fibrotic control glomeruli; and the ATS-treated, drug treated, fibrotic glomeruli to determine the degree to which the fibrotic process is inhibited by somatostatin or the somatostatin agonists.

(b) Demonstration of Anti-Fibrotic Activity *In Vivo*

Rats are injected either with anti-thymocyte serum (ATS) to induce glomerulonephritis or with phosphate buffered saline (PBS) served as controls. One hour later, treatment is initiated with somatostatin or the somatostatin agonists. Somatostatin or the somatostatin agonists are administered subcutaneously once per day for 5 days. On day 5, the rats are placed in metabolic cages, and 24 hour urine is collected to determine protein content. On day 6, the kidneys are removed, and tissue samples are either placed in formalin or frozen for histological evaluation. Glomeruli are isolated from the remaining tissue and are placed in culture for 72 hours. Culture conditions consisted of 2000 glomeruli/well in a 1 ml volume of serum-free RPMI 1640 (with insulin supplementation). The supernatant from the cultures are collected and stored at -70ºC until assayed to determine the concentration of collagen I, transforming growth factor β-1 (TGFβ-1 fibronectin containing an extra domain A (fibronectin EDA+), and plasminogen activator inhibitor I (PAI-I) as markers of fibrotic activity. In addition, individual glomeruli are examined by immunofluorescent staining and scored for relevant matrix proteins. Values are compared between PBS-treated, negative fibrotic control animals; ATS-treated, non-drug treated, positive fibrotic control animals, and the ATS-treated, drug-treated animals to determine the degree to which the fibrotic process is inhibited by somatostatin or the somatostatin agonist.

Other Embodiments

The foregoing description has been limited to specific embodiments of this invention. It will be apparent, however, that variations and modifications may be made to the invention, with the attainment of some or all of the advantages of the invention. Such embodiments are also within the scope of the following claims.

What is claimed is:

1. A method of inhibiting fibrosis in a patient said method comprising administering a therapeutically effective amount of somatostatin or a somatostatin agonist to said patient.

2. A method of claim 1, wherein said method comprises administering a therapeutically effective amount of a somatostatin agonist to said patient.

3. A method of claim 2, wherein said fibrosis is in the kidney.

4. A method of claim 2, wherein said fibrosis is in the lung.

5. A method of claim 2, wherein said fibrosis is in the liver.

6. A method of claim 2, wherein said fibrosis is in the skin.

7. A method of claim 2, wherein said fibrosis is induced by chemotherapy.

8. A method of claim 2, wherein said somatostatin agonist is administered parenterally.

9. A method of claim 8, wherein said somatostatin agonist is administered in a sustained release formulation.

10. A method of claim 3, wherein said somatostatin agonist is administered parenterally.

11. A method of claim 10, wherein said somatostatin agonist is administered in a sustained release formulation.

12. A method of claim 4, wherein said somatostatin agonist is administered parenterally.

13. A method of claim 12, wherein said somatostatin agonist is administered in a sustained release formulation.

14. A method of claim 5, wherein said somatostatin agonist is administered parenterally.

15. A method of claim 14, wherein said somatostatin agonist is administered in a sustained release formulation.

16. A method of claim 6, wherein said somatostatin agonist is administered parenterally.

17. A method of claim 16, wherein said somatostatin agonist is administered topically.

18. A method of claim 7, wherein said somatostatin agonist is administered parenterally.

19. A method of claim 18, wherein said somatostatin agonist is administered in a sustained release formulation.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/08999

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00; C07K 14/00
US CL :514/12, 14, 806; 530/311
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 14, 806; 530/311

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
somatostatin analog, fibrosis, treat, or inhibit, cirrhosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TSUKAMOTO et al. Octreotide Treatment Results in the Inhibition of GH Gene Expression in the Adenoma of the Patients with Acromegaly. Endocrine Journal. 1994, Vol. 41, No. 4, pages 437-444, see entire document.	1, 2
X	TRACY et al. Somatostatin Analogue (Octreotide) Inhibits Bile Duct Epithelial Cell Proliferation and Fibrosis After Extrahepatic Biliary Obstruction. American Journal of Pathology. December 1993, Vol. 143, No. 6, pages 1574-1578, see entire document.	1, 2
Y	US 4,904,642 A (COY et al.) 27 February 1990, see column 2, lines 17-20; column 4, lines 21-25.	1, 2, 5, 8-9, 14, 15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 JULY 1997	06 AUG 1997
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